Agricultural Research Service • National Program 304 • Crop Protection and Quarantine
FY 2016 Annual Report

The Crop Protection and Quarantine National Program (NP 304) addresses high priority insect, mite, and weed pest problems of crops, forests, urban trees, rangelands, postharvest systems (such as stored grains), and natural areas.

U.S. agriculture provides the Nation with abundant, high quality, and reasonably priced food and fiber. From corn and cotton, potatoes, peanuts, pumpkins, and peas to apples, alfalfa, almonds, soybeans, citrus, nuts, berries, and beans, American agriculture annually plants over a quarter of a billion acres of food and fiber crops worth over $115 billion. Additionally, agricultural commodities represent about six percent of the total value of all our domestic exports. Economic losses of our food and fiber due to insects, mites, and weeds, however, are considerable, with estimates in the tens of billions of dollars. Pest control includes cultural, biological, physical, and chemical methods. Non-chemical methods based on biological knowledge continue to expand, but the Nation continues to depend heavily on chemical control to produce agricultural commodities. Maintenance of our arsenal of valuable agricultural chemicals is a constant challenge as we lose ingredients to resistance, new regulatory requirements related to public acceptance, and due to commercial considerations. Furthermore, the problem of losses due to insect pests does not end in the field or with the harvest. Insects reduce the quality of stored grain and other stored products, and it is estimated that postharvest losses to corn and wheat alone amount to as much as $2.5 billion annually. Imported commodities as well as those destined for export must be protected from native and exotic pests. Exotic insect and weed pests that threaten our food, fiber, and natural ecosystems are another mounting concern due to the increase in world trade and travel. Invasive species directly threaten our agricultural crops, transmit devastating bacterial and viral diseases that threaten entire agricultural industries, and decimate our forests and urban landscapes; while invasive weeds reduce biodiversity, displace native species, and cost billions of dollars to control.

The goals of NP 304 are twofold: to understand the biology, ecology, and impact of these pests on agricultural production and natural systems and to develop, improve, and integrate environmentally safe technologies to exclude, eradicate, or manage pest populations. Priority is placed on sustainable and integrated practices that enhance the productivity, quality, and safety of U.S. agriculture while protecting natural resources, native ecosystems, human health, and the environment.

This National Program is divided into four research components:

- **Component 1: Systematics and Identification:** accurately identifying insects, mites, and weeds, whether native or invasive, to get important information about their possible country of origin and bionomics, and the taxonomy and systematics of microorganisms associated with these insects and weeds, for aid in developing microbials as biological control agents
- **Component 2: Protection of Agricultural and Horticultural Crops:** improving existing and/or developing new, innovative control strategies for pests in traditional and organic agricultural and horticultural systems
- **Component 3: Protection of Natural Ecosystems:** preventing, managing, and controlling critical insect pests and weeds that threaten environmental areas and the agricultural areas bordering them
- **Component 4: Protection of Postharvest Commodities and Quarantine:** contributing to the development of effective and sound management strategies to reduce pest damage that occurs after harvest, to limit the spread of exotic pests on agricultural commodities, and to ensure U.S. competitiveness in the international commerce of agricultural commodities

Below are research accomplishments for this national program from fiscal year 2016. The results are presented under the components and problem statements of this program’s 2015-2020 Action Plan. The report below is not intended to be a progress report describing all research conducted during the 2016 fiscal year; rather it is an overview that highlights major accomplishments, some of which are based on multiple years of research (not all research projects will reach an "accomplishment" endpoint each year).

ARS welcomes your input regarding our ongoing research programs. If you have any questions, please do not hesitate to contact the co-leaders of National Program 304, Kevin Hackett (Kevin.Hackett@ars.usda.gov) and Rosalind James (Rosalind.James@ars.usda.gov).

Component 1 – Systematics and Identification

Identified tolerance to Prunus replant disease in rootstocks for almond and other stone fruits. Cultivated species of *Prunus* include almonds, peaches, and other stone fruits covering more than a million acres in California alone. Orchards must be replanted every 15 to 25 years to remain economical. Preplant soil fumigation is practiced widely among replanted orchards to control Prunus replant disease (PRD), a growth suppression induced by a crop-specific soilborne microbial complex in the soil. PRD affects Prunus species planted after closely related species. ARS scientists in Davis, California, conducted replant trials to identify rootstocks with tolerance to PRD. Among 20 rootstock selections tested, all rootstocks were impacted by PRD, but peach x almond hybrid rootstocks were impacted much less severely than peach rootstocks. Although peach x almond hybrid rootstocks are not appropriate for poorly drained soils or those infested with certain plant parasitic nematodes, their use may reduce the need for preplant fumigation in well-drained soils impacted only by PRD.

Component 2 – Protection of Agricultural and Horticultural Crops

Herbicide resistance in yellow nutsedge, a major weed in rice production. Yellow nutsedge is one of the most problematic weeds in Arkansas rice requiring frequent herbicide use of a product called halosulfuron (sulfonylurea) for control. In 2012, halosulfuron failed to control this weed at the field rate specified on the product label. ARS scientists from Stoneville, Mississippi, in collaboration with scientists from the University of Arkansas and Auburn University, examined the resistance level, cross-resistance and resistance mechanism in this putative resistant biotype. Resistant nut sedges were not killed by halosulfuron at a dose 256-fold higher than the field dose, nor were they killed by acetolactate synthase (ALS)-inhibiting herbicides (imazamox, imazethapyr, penoxsulam, bispyribac, pyrithiobac-sodium, bensulfuron and halosulfuron) at labeled field rates. The ALS enzyme from the resistant biotype was 2540-fold less responsive to halosulfuron than a susceptible biotype, and resistance appears to be caused by a mutation creating a single amino acid substitution in the ALS enzyme. In other words, a single, target-site mutation conferred a high degree of resistance in yellow nutsedge, and conferred cross-resistance to other ALS-inhibiting herbicide families.

Goss's wilt incidence in sweet corn is independent of the use of the herbicide glyphosate and transgenic traits. Claims have been made in recent years that corn susceptibility to plant diseases, including Goss's wilt, increases with the use of glyphosate use and transgenic traits. ARS researchers in Urbana, Illinois, and Beltsville, Maryland, in collaboration with scientists from the University of Mississippi, found that Goss's wilt incidence was independent of glyphosate use and transgenic traits in sweet corn, and furthermore sweet corn yield improved with the use of these technologies. This research dispels unsubstantiated, negative claims about the effects of glyphosate and transgenic traits on corn.

National fungal collection established for endophytes associated with invasive plants. Fungi that live symbiotically within living plants are called endophytes, and research has shown they can benefit crop plants. However, the role endophytes play in the success of invasive weeds is not well known. ARS scientists in Peoria, Illinois, successfully collected, identified, cultured, and stored over 350 fungal isolates from medusahead rye. This annual grass is invading the far west, displacing native grasses and driving wildfires that are destroying the re-establishment of habitat for sage-grouse, a native bird in rapid decline. The invasive plant endophyte culture collection will serve as a critical resource for studying symbiotic interactions that drive plant invasions. This collection will support the ultimate research goal of developing environmentally sound weed control strategies.

New bio-based weed inhibitors found in toothpick weed. Plants constitute a rich source of novel and diverse compounds to be explored for effective and environmentally safe herbicides. ARS scientists in Oxford, Mississippi, found two highly phytotoxic compounds, khellin and visnagin, in toothpick weed. In laboratory tests, these compounds inhibited the growth of the weeds, duckweed, weedy ryegrass, morning glory, foxtail and weedy millet, as well as lettuce, with similar or greater efficacy than the commercial herbicides acetochlor and glyphosate. In addition, visnagin was tested also in the greenhouse on velvetleaf, crabgrass and barnyardgrass, with good success. These compounds appear to have a unique mode of action. Thus, visnagin, and possibly khellin, are good candidates for further development into bioherbicides, or as lead molecules for the development of new herbicides.

Hairy vetch and cereal rye cover crop mixtures improve nutrient management and weed suppression. Legume cover crops provide nitrogen to the subsequent cash crop, while grasses provide good weed control and scavenge soil nitrogen. To best make recommendations to farmers on how to use cover crop mixtures, we must understand how soil and crop management practices (fertilizer and tillage) influence the cover crop once terminated. Therefore, ARS researchers in Beltsville, Maryland, conducted an experiment at two locations for two years to assess how seeding rates, tillage, and poultry litter applications (broadcast vs. subsurface banded) influence cover crop decomposition. ARS found that maximum residue persistence can be achieved using a pure cereal rye cover crop combined with either no poultry litter, or subsurface banded poultry litter. Pure hairy vetch residue released the most nitrogen, particularly when incorporated with poultry litter; however, incorporation also sped up the rate of nitrogen release, making it less synchronous with the nitrogen needs of a corn cash crop. These results will be used to develop web-based decision support tools for growers, aiding in the development of optimal nitrogen management.

Improved weed management in sugar beet grown for biofuel. Sugar beet, grown for biofuel as energy beet, is being considered as an alternate cool-season crop in the southeastern U.S. coastal plain. Weed control must be cost-effective to make this alternative cropping system feasible. Herbicides registered for use on sugar beet are costly. ARS researchers in Tifton, Georgia, effectively controlled the most troublesome weed of sugar beet, cutleaf evening primrose, with sweep cultivation, and reduced rates of the herbicides phenmedipham plus desmedipham. This integrated approach to managing weeds provided adequate control of weeds, protected sugar beet yields, and reduced costs by >30 percent. This unique cropping system will give growers an option to efficiently utilize crop land during the winter growing season in the southeastern U.S.

Component 3 – Protection of Natural Ecosystems

New technology developed to control the gypsy moth. Gypsy moth is a voracious and rapidly spreading insect pest that defoliates millions of acres of hardwood forest annually, and often plagues the nation’s urban greenscapes. Using their gypsy moth transcriptomes, ARS scientists in Beltsville, Maryland, used recently developed technologies to create RNA interference (RNAi)-based molecular biopesticides that target specific genes crucial to the survival of the moth. Several novel dsRNAs were specifically designed to deactivate and silence these genes, negatively impacting the normal physiology of the insect. These compounds, when fed to the caterpillars, reduced their reproductive capacity. These new and highly-specific biopesticide technologies will be useful tools for controlling gypsy moth pests, while sparing non-target insects.

RNA interference (RNAi)-based molecular biopesticides effective against brown marmorated stink bug (BMSB). The brown marmorated stink bug (BSMB), an invasive insect native to Asia, has rapidly emerged as a major insect pest in the United States. Well known to the public as an irritating indoor nuisance pest, BMSB is a polyphagous piercing/sucking feeder that poses considerable ecological and economic threat to specialty crops, including apples, stone and pome fruits, grapes, ornamental plants, vegetables, and seed crops, as well as staple crops such as soybean and corn. ARS scientists in Beltsville, Maryland, designed novel double-stranded RNAs to deactivate and silence specific genes critical to BMSB. When fed to BMSB, these compounds inhibited development and reduced fecundity. These new and highly-specific molecular biopesticide technologies will be useful tools for controlling BMSB pests without affecting non-target insects.

A new tool delivered for fight against citrus greening disease. Bactericide application technology became available to Florida citrus growers in 2016 to fight citrus greening disease. ARS researchers at Fort Pierce, Florida, demonstrated that bactericide formulations designed to penetrate citrus trees and reach the phloem reduced bacterial titer and improved tree health. Data provided to the State of Florida and federal Environmental Protection Agency were the basis for approval of these bactericides for use in Florida. It is estimated they are now being used on the majority of Florida's commercial citrus orchards.

Codling moth control made more economical. Codling moth is a key pest of apple, pear, and walnuts in the United States and elsewhere in the world. Mass trapping with lures that attract female moths, can replace pesticide applications and augment mating disruption. ARS scientists in Wapato, Washington, developed an inexpensive sachet system that optimized a controlled release of acetic acid, a fruit volatile, and pear ester as a lure for female and male codling moths. Field tests validated the attractiveness and performance of the new dispenser system as a lure for traps. Codling moth damage can be controlled using 50 of these baited traps per acre, thus lowering the costs for lures and traps significantly lowering overall control costs. These developments show promise as new technologies for the control of codling moth by conventional and organic fruit growers.

Wild potato germplasm resistant to potato psyllid. The potato psyllid is the vector for the pathogen associated with zebra chip disease of potato that renders potato tubers unmarketable. Wild potato germplasm provides genetic sources of desirable traits, including insect resistance that can be bred into marketable potato cultivars. ARS researchers in Wapato, Washington, and in Sturgeon Bay, Wisconsin, screened populations of a wild potato species, *Solanum verrucosum,* for resistance to potato psyllid. They discovered two populations that are highly resistant to potato psyllid. These two populations will be used by breeders to develop new cultivars that are resistant to potato psyllid, providing a cost-effective method of control of the potato psyllid and zebra chip pathogen without the use of insecticides.

Reduction of optimal thermal range in aging western cherry fruit flies. The potential of Western cherry fruit fly to invade new geographic areas is thought to be limited by intolerance to extreme temperatures, resulting in a shutdown of metabolic activity and development. This can be measured by its oxygen consumption and metabolic rate. ARS scientists in Wapato, Washington, determined that the fly's tolerance of temperature extremes is impacted by the age of the fly. Newly emerged flies showed the broadest tolerance to temperatures, maintaining metabolic activity from 6.6 to 42.2 degrees Celsius (a range of 35.8 degrees); 28-day old flies showed the shortest range of temperatures suitable for metabolic activity from 10.5 to 37.8 degrees Celsius (a range of 27.2 degrees). These results were used to refine a model to predict the potential distribution of this pest nationally and globally, which in turn is being used by the northwest sweet cherry industry to renegotiate current and establish new trade agreements, where quarantine restrictions exist against the western cherry fruit fly but models show no risk of invasion.

Compendium of Fruit Fly Host Information (CoFFHI) updated. The Compendium of Fruit Fly Host Information has been released online to provide centralized, comprehensive, interactive and searchable documentation of what is known, worldwide, about the status of fruits and vegetables as hosts of fruit flies of economic importance. Fruit flies cause direct damage to fruits and vegetables through oviposition and larval feeding and restrict movement of commodities across national and international borders. Establishment of appropriate regulatory procedures, however, is dependent on the knowledge of the status of commodities as hosts for fruit fly species. The online accessible Compendium of Fruit Fly Host Information (CoFFHI; https://coffhi.cphst.org/), developed through collaborative efforts of ARS scientists in Hilo, Hawaii, the USDA Animal and Plant Health Inspection Service, the Center for Plant Health Science and Technology (APHIS-CPHST in Raleigh, North Carolina), and the Center for Integrated Pest Management (CIPM in Raleigh, North Carolina) provides comprehensive documentation of host records for the Mediterranean fruit fly, the carambola fruit fly, the guava fruit fly and Bactrocera latifrons, as well as updated host lists for the oriental fruit fly and the melon fly. As a primary reference on fruit fly host plants, CoFFHI is designed to enable regulatory scientists and regulatory officials to assess and mitigate risk of introduction and establishment of exotic fruit flies, in fresh horticultural commodities, that pose significant threats to U.S. agriculture and natural resources. It also serves as a decision tool in the design and implementation of effective fruit fly detection, monitoring, suppression, and eradication programs of USDA and various state regulatory agencies.

Development of a vibration trap and mating disruption systems for Asian citrus psyllids. Asian citrus psyllids (ACP) are an invasive insect pest in Florida citrus groves and the arthropod vector of the bacterium that causes citrus greening. Unlike most insects, male and female ACP use wing-buzzing vibrations instead of pheromones to locate and court one another in citrus trees. Researchers at ARS in Gainesville, Florida, developed and tested a highly efficient device that mimics female-produced courtship vibrations to trap male ACP. As an extension of this research, vibrational signals that mimic females to disrupt mating were also tested. Tests in the laboratory have proven successful and devices are being engineered for the transfer of this technology to field environments to improve detection and control of ACP populations.

Effective delivery of a novel pest control compound. Insect pests have developed resistance to several conventional pesticides, and new approaches that target critical life processes are needed for pest management. Although neuropeptides (short chains of amino acids) serve as potent messengers in insects to regulate vital functions, the neuropeptides themselves hold little promise as pest control agents because they can be degraded in the target pest and fail to efficiently penetrate the outside barrier of insects. New selective control agents may be developed by designing mimics of these neuropeptides that both resist degradation and show greater penetrability, which in turn, then either inhibits or over-stimulates critical neuropeptide-regulated life functions. One such life function is the dormant state known as diapause that is widely exploited by insects to circumvent winter and other adverse seasons. ARS researchers at College Station, Texas, in collaboration with scientists from the Ohio State University, developed novel versions of neuropeptides of the ‘Diapause Hormone’ class that, for the first time, penetrate the outer barrier of pupae of heliothine insects (e.g., corn earworm, cotton bollworm, and tobacco budworm), preventing them from entering the protective state of diapause and inducing them to commit a form of ‘ecological suicide’. This work represents a major breakthrough in the development of a completely new, practical, and environmentally friendly strategy based on neuropeptide-like substances for control of pest insects via disruption of diapause.

Discovery of a new emerald ash borer biocontrol agent. The emerald ash borer (EAB) is a serious invasive forest pest that has been detected in 27 U.S. states and killed hundreds of millions of ash trees since it was first detected in Michigan in 2002. Classical biocontrol via introduction of a diverse complex of co-evolved natural enemies from the pest's native range (Northeast Asia) may be the only viable option for effective long-term management of EAB in North America natural forests. ARS researchers in Newark, Delaware, discovered and described a new species of EAB egg parasitoid previously collected from the Russian Far East. This species is new to science and described as *Oobius primorskyensis*. Further laboratory study reveals that this new species shows distinctive differences in the reproductive biology and progeny dormancy pattern from the previously introduced EAB egg parasitoid *(O. agrili)* and may be more suitable for introduction against EAB in Northeast U.S. This new egg EAB parasitoid is currently being evaluated by USDA-APHIS for environmental release as a biocontrol agent and may help improve the efficacy of the current EAB biocontrol programs leading to survival and recovery of North American ash trees.

Completed whole genome sequencing, assembly and annotation of the Mediterranean fruit fly. The Mediterranean Fruit Fly (Med Fly), is one of the most serious agricultural threats to U.S. agriculture and throughout the world. The Med fly genome was sequenced and annotated in order to obtain extensive knowledge of its genetic pathways essential to development, behavior and reproduction. Researchers at ARS in Gainesville, Florida, and Hilo, Hawaii, along with an international assemblage of academic colleagues, collaborated to conduct the sequencing and an in-depth manual curation of the genome and transcriptome. They identified specific genetic expansions that can be related to invasiveness and host adaptation, including chemoreception, toxin and metabolism, immunity, and cuticular proteins. This new knowledge, will provide novel genetic tools and advanced technologies for controlling Med Fly populations, such as new tools to enhance control by the Sterile Insect Technique (SIT).

Development of biologically-based control for spotted wing drosophila. Non-toxic insecticides, especially those available for organic growers, are being sought for control of a small fruit and cherry pest called the spotted wing drosophila (SWD). ARS scientists in Corvallis, Oregon, found that the non-nutritive sweeteners, erythritol and erythrose, have insecticidal effects against SWD adults in laboratory studies. These artificial sweeteners were dissolved in water and fed to SWD adults, which resulted in more than 60% mortality within 4 days. Thus these two sweeteners have potential as organic insecticides.

Pesticide residue levels in specialty food crops and ornamental plants. The primary entity in the United States to facilitate registrations of conventional pesticides and biopesticides on specialty food crops (fruits, vegetables, nuts) and non-food ornamental horticulture crops (greenhouse flowers, nursery, landscape plants, and Christmas trees) is the Interregional Research Project Number 4 (IR-4). ARS researchers at Wapato, Washington, in collaboration with IR-4 personnel at Rutgers University in New Jersey, tested new candidate pesticides on 10 food crops, evaluating pesticide residues and efficacy against pests. These pesticides were also tested on 12 ornamentals to evaluate crop safety. Most of the pesticide residues on the food crops were below the minimum levels deemed safe by the Environmental Protection Agency (EPA), and thus these products are safe to use on minor crops. This information will be submitted to EPA by the IR-4 Project to enable registrations of pesticides needed to protect minor food crops and ornamental plants from insect pests.

Component 4 – Protection of Postharvest Commodities and Quarantine

Identification of new attractant for the shot hole borer, an invasive pest of trees. While conducting research on attractants for red ambrosia beetles in avocado groves, ARS scientists in Miami, Florida, discovered that alpha-copaene attracts the shot hole borer, *Euwallacea* nr. *fornicatus*. The shot hole borer transmits Fusarium dieback, a serious plant disease affecting the avocado industry in California. The shot hole borer was first detected in Florida groves in 2012, at low numbers and with little economic impact; however, beetle numbers are increasing in Florida, accompanied by tree damage comparable to that in

California. Research by ARS scientists indicates that alpha-copaene is equal in attraction to the current shot hole borer lure (quercivorol), but a combination of the two chemicals is synergistic, resulting in significantly higher beetle capture. Ongoing research will further evaluate efficacy and longevity of this new two-component lure for shot hole borer. In collaboration with the University of Florida and Florida avocado growers, the new lure is also being used to survey the avocado production area of Miami-Dade County to determine the prevalence of this new pest.

Fruit fly control allows Hawaii to export Sharwil avocados. Sharwil avocados produced in Hawaii are known for their large size and buttery taste, but were banned from entry into the mainland U.S. in 1992 after a fruit fly species was found in an outbound shipment. During this time, the U.S. per capita consumption of avocados in general has increased nearly seven-fold, out pacing mainland production levels. Hawaiian avocados could still be shipped if they went through a fumigation process, but that can affect taste. ARS scientists in Hilo, Hawaii, developed a systems approach for fruit fly control in avocados that was so effective, it has been approved by the Animal Plant Health Inspection Service (APHIS) as an export protocol. The first shipment of Hawaiian Sharwil avocados, in 25 years, was made this year (951 pounds were sent to Minneapolis, Minnesota), and several growers are planting new avocado orchards to take advantage of the export program. This pest control system will help increase U.S. contributions to domestic demand for avocados.

Genome of the lesser grain borer sequenced and annotated. The lesser grain borer has immature stages that feed internally in grain, and thus are difficult to control. In addition, grain borer populations are becoming resistant to many commonly used control methods, including phosphine fumigation. Genome sequence information is needed to understand genes that underlie basic biological functions in insects including insecticide resistance. However, currently only the genome for one grain pest, the red flour beetle, has been sequenced. ARS scientists in Manhattan, Kansas, used data from short read sequencing platforms (Ion Torrent PGM, Illumina Mi-Seq) and long read platforms (PacBio) to begin sequencing the genome of the lesser grain borer. Genes have been predicted and annotated in the draft genome with sequencing data from phosphine-resistant insects be used to map resistance genes and identify pathways that contribute to resistance. Using this approach, current resistance management strategies for improved efficacy will be modified, as well as development of new probes to monitor for resistance in the field.

Nitric oxide fumigation treatment for control of light brown apple moth. Light brown apple moth is quarantined in most countries and an effective treatment is needed to facilitate exports of U.S. fresh products. An ARS researcher in Salinas, California, successfully controlled light brown apple moth with nitric oxide fumigation. Nitric oxide was developed as a new fumigant at USDA-ARS and is effective against a wide variety of pests. Nitric oxide fumigation under ultralow oxygen conditions achieved complete control of light brown apple moth eggs, larvae, and pupae at a low cold storage temperature in 6 to 48 h depending on nitric oxide concentrations. The study demonstrates the great potential of nitric oxide fumigation in controlling postharvest pests including light brown apple moth on exported fresh products.

Developing new technology to ensure compliance with maximum residue levels for export crops. The export of California tree nuts to the European Union has an estimated value of $2.5 billion annually. An ARS scientist in Parlier, California, developed novel analytical methodologies to quantify and decrease levels of pesticide residues on tree nuts in order to comply with the maximum residue levels for almonds and walnuts established by the European Union. These methodologies included the use of gas chromatography-mass spectrometry to quantify residues of phosphorous acid as well as development of a new method to quantify residues of propylene oxide and its halohydrins. The transfer of this method to European Union chemists and regulators directly resulted in the establishment of temporary phosphorous acid maximum residue levels for tree nuts through March 2019, thereby preserving the export of California tree nuts until a permanent import tolerance can be established. This research also serves as a key basis for technical interaction between California tree nut industry, USDA-Foreign Agricultural Service, USDA-Animal and Plant Health Inspection Service, U.S. Environmental Protection Agency, and respective counterparts in foreign governments, with the ultimate goal of protecting export markets.